# Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatments

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*Received 17 September 1993*

*Small numbers of* Listeria monocytogenes *can be isolated from packaged frankfurters. Based on previous work from this laboratory, these* L. monocytogenes *cells undoubtedly represent surface recontamination during peeling and packaging. We investigated organic acid dips just prior to packaging as a secondary lethal step to destroy* L. monocytogenes *and observed both bacteriocidal and bacteriostatic effects depending on the concentration of acid used. Acetic acid when combined with citric acid (at 2·5% each) restricted the growth and development of* L. monocytogenes *on frankfurters stored vacuum-packaged at 5°C for up to 90 days. Other acids individually, such as lactic acid (5%) and acetic acid (5%), also suppressed the organism. A dose response effect was observed for all acids tested. A simple 2 min surface rinse in saline was adequate to remove* L. monocytogenes *from frankfurter surfaces and gave recoveries similar to a stomacher method. The acid treatments appeared to be lethal and did not injure the organism. Overall, organic acid treatments appear to provide a secondary lethal step in frankfurter processing and could be an additional factor in limiting the presence of* L. monocytogenes *in frankfurters.*

## Introduction

*Listeria monocytogenes* is a Gram-positive, psychrotrophic bacterium which occurs widely in nature, specifically on vegetation, water and ultimately in various foods such as red meats (Johnson et al. 1990), poultry, fish and seafood, and raw milk (Buchanan et al. 1989).

Recent studies on the control of the organism have investigated the ability of different treatments to destroy the organism during processing: raw milk (Lovett et al. 1989), ice cream mix (Holsinger et al. 1992), processing of pork-beef frankfurters (Zaika et al. 1990) and liver sausage processing (Palumbo et al. 1993).

However, *L. monocytogenes* can be isolated from products which have received a lethal, treatment. For example, the organism has been isolated from ice cream products, turkey frankfurters (Wenger et al. 1990), and other processed, ready-to-eat meats (Johnson et al. 1990). The presence of *L. monocytogenes* in these products undoubtedly reflects recontamination of the food after processing and before packaging. Previous work from this laboratory has indicated that *L. monocytogenes* can survive for long periods of time under

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conditions similar to those encountered in meat processing plants; these surviving cells could serve as a source of recontamination of properly heated products (Palumbo and Williams 1990). The development of a secondary, lethal treatment for the organism would provide additional protection and safety for the consumer and processor. Organic acid dips and sprays have proved useful in increasing the bacteriological shelf-life of various foods such as red meats, poultry, etc. (Anderson and Marshall 1989, 1990, Mendonca et al. 1989, Surve et al. 1991, Zeitoun and Debevere, 1991, Prasai et al. 1992) In this study, we have evaluated post-heat processing organic acid dips of a processed meat product, frankfurters, to control the presence of any *L. monocytogenes* which might contaminate their surfaces after peeling and before packaging.

## Materials and Methods

### *Micro-organisms*

A mixture of the following strains of *L. monocytogenes* (originally obtained from FDA, Cincinnati, OH and FSIS, Beltsville, MD, USA) was used to inoculate the surface of the frankfurters: RMI, RMII, ATCC 7644, V7, Scott A, Murray B and V97. The strains were grown individually at 37°C for 18–20 h aerobically in BHI Broth (Difco, Detroit, MI, USA) with 0·3% glucose added. One ml of each culture was then added to 500 ml sterile distilled water and mixed thoroughly; the frankfurters were then immersed for 2 min in this cocktail of strains.

### *Frankfurters*

Standard pork-beef frankfurters (30% fat) were obtained unpeeled from a local processor. Upon arrival at the laboratory, they were peeled manually in a manner to minimize contamination. In one experiment, a brand of commercial frankfurters previously shown to have extremely low background counts and no detectable *L. monocytogenes* (by direct plating) was purchased in a local supermarket and again handled in a manner to minimize recontamination. The frankfurters from the local processor also had extremely low background counts and no detectable *L. monocytogenes*.

### *Acid treatment*

After the frankfurters were dipped in the culture cocktail, they were either drained and vacuum-packaged, or drained, immersed for 2 min in an acid solution, drained and vacuum-packaged. The acid solutions (in distilled water) were (v/v or w/v): acetic, lactic, tartaric, citric or a mixture of acetic and citric. The concentration of acid in the dip was varied and is specifically indicated for each experiment. The acids were reagent grade and were used directly as received from the manufacturer. In one experiment, the order was reversed and frankfurters were acid dipped and then exposed to the culture cocktail. Preliminary experiments indicated that only small numbers (< 1%) of *L. monocytogenes* were removed when the inoculated frankfurters were dipped for 2 min in distilled water alone.

### *Packaging and storage*

After the various treatments, the frankfurters were vacuum-packaged (SuperVac, Smith Equipment Co., Clifton, NJ 07012; Koch Industry bags, 3 mil, code 01-46-09, Kansas City) and stored at either 5 or 12°C.

### *Bacteriology*

To prepare the frankfurters for viable *L. monocytogenes* determinations, they were aseptically removed from the packages and placed in Stomacher Filter Bags (Spiral Systems, Bethesda, MD) with four weight volumes of sterile physiological saline (8·5 g/l NaCl). The frankfurters then were agitated by hand and allowed to stand for 2 min to remove the organisms adhering to their surfaces (surface rinse); any further dilutions were prepared as needed in 0·1% peptone water. After an aliquot was removed for Spiral plating, the frankfurter-peptone water was mixed for 2 min in a Stomacher 400 laboratory mixer (Dynatec); the filter bag was then removed and the number of viable *L. monocytogenes* determined on the remaining suspension (stomacher treatment) by preparing dilutions as needed in 0·1% peptone water followed by plating.

At weekly or biweekly intervals, the number of viable *L. monocytogenes* was determined by surface plating onto various media using a model D spiral plater (Spiral Systems,

**Table 1. Effect of variation of acid treatment on the recovery of *L. monocytogenes* on the surface of frankfurters (TA vs TAS): increased exposure time and exposure to higher concentrations of acid (recovery by surface rinse technique).**

| Treatment | TA[a] | TAS[a] |
|---|---|---|
| Lm culture only | 4·71[b] | 4·51 |
| Lm culture then 2% lactic acid for different lengths of time: | | |
| 1 min | 3·69 | 3·68 |
| 2 min | 3·50 | 3·41 |
| 3 min | 3·04 | 3·29 |
| 4 min | 3·10 | 2·88 |
| 5 min | 3·38 | 3·11 |
| Lm culture then different concentrations of acid for 2 min: | | |
| 1·5% acetic acid | 4·08 | 3·93 |
| 3·0% acetic acid | 3·83 | 3·88 |
| 4·5% acetic acid | 3·50 | 3·44 |
| 1·5% lactic acid | 3·61 | 3·50 |
| 3·0% lactic acid | 3·08 | 3·04 |
| 4·5% lactic acid | 2·74 | 2·55 |

[a]TA, tryptose agar; TAS, tryptose agar + 5% NaCl; the difference between the two is a measure of the amount of injury.
[b]$\log_{10}$ viable *L. monocytogenes* $g^{-1}$ frankfurter, average of duplicate plates of duplicate frankfurters.

Spiral Biotech, Bethesda, MD). The following media were used to quantitate *L. monocytogenes*: tryptose agar (TA; Difco, Detroit, MI), TAS (TA + 5% NaCl; Smith and Archer 1988), and MVJ (modified Vogel Johnson agar; Buchanan et al. 1989). Plates were incubated aerobically at 37°C for 48 h and then total colonies on TA and TAS and black colonies on MVJ were counted. Periodically during various experiments, the possible presence of lactic acid bacteria was assessed by surface plating onto Rogosa SL agar (Difco); these plates were incubated aerobically at 28°C and examined after 3 days.

### *Analysis of data*

The viable plate count data were converted to $\log_{10}$. The Student's *t*-test was used in a Lotus 123 (version 2.2) worksheet to compare the various treatments (Snedecor and Cochran 1980). Treatment responses (Table 1) were analyzed by linear regression using the Lotus 123 regression data function.

## Results and Discussion

The system described here, using unpeeled commercial frankfurters or one specific brand of frankfurters from a local supermarket, provided a very satisfactory one for assessing the effect of organic acid treatments to inhibit *L. monocytogenes* on frankfurter surfaces. *L. monocytogenes* were never detected on the control (uninoculated) frankfurters. Background microflora were seldom detected on the controls and then at such low levels so as not to interfere with detecting *L. monocytogenes* on any of the treated frankfurters. Lactic acid bacteria were rarely detected, and then only at very low levels.

### *Destruction of* L. monocytogenes *on the surface of frankfurters by acid treatments*

In this initial experiment, we compared a two min dip in 1% solutions of different organic acids for their ability to destroy *L. monocytogenes* on the surface of frankfurters (Fig. 1). At the time of treatment (zero time of storage), there was a significant difference in count ($P > 0·95$) between the count of the

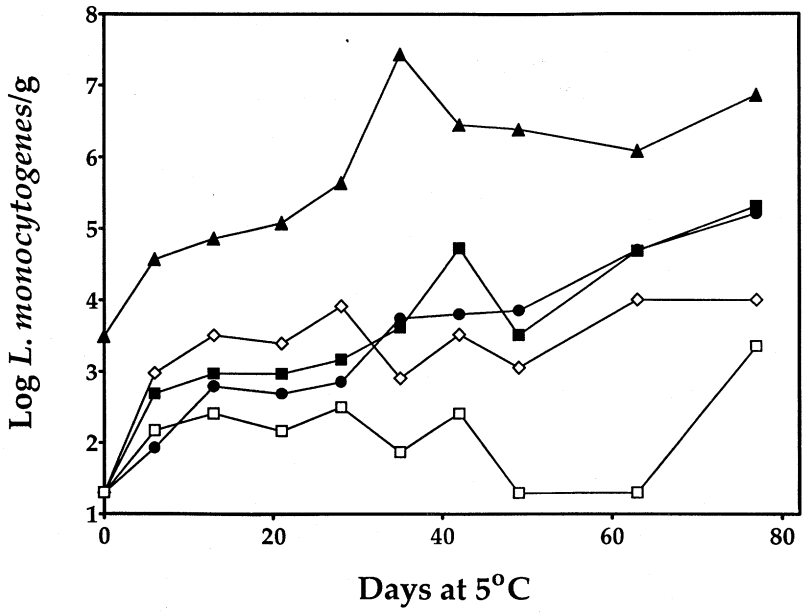


**Fig. 1.** The effect of acid treatment [2 min dip in an *L. monocytogenes* culture (cocktail of 7 strains) followed by a 2 min dip in 1% solution of various organic acids] on the growth of *L. monocytogenes* on the surface of frankfurters stored at 5°C. Points represent the average of duplicate plates of duplicate frankfurters; *L. monocytogenes* recovered by surface rinse; plated on MVJ. A count of 1·3 is at or below the lower limit of detection. ▲, Lm culture; ●, Lm culture and tartaric acid; ◇, Lm culture and acetic acid; ■, Lm culture and citric acid; □, Lm culture and mixture of 0·5% acetic and 0·5% citric acids.

inoculated, untreated frankfurters and those treated with, the organic acids. While *L. monocytogenes* grew readily on untreated product, growth was slower on acid dipped frankfurters, particularly those dipped in the combination of acetic and citric acids. Other investigators have also observed the ready growth of *L. monocytogenes* on frankfurters and frankfurter-type products when held vacuum-packaged at 2–7°C (Buncic et al. 1991, Glass and Doyle 1989, Schmidt and Kaya 1990). The extent of increase in numbers described by these investigators depended on storage temperature.

*Processing of samples: surface rinse vs stomacher treatment*

In this experiment, we compared both methods of recovering *L. monocytogenes* from inoculated frankfurters and inoculated-acid treated (2% acetic acid vs 1% each citric and acetic acids). The recovery data are presented in Fig. 2 and were analyzed by the *t*-test. The recovery of *L. monocytogenes* from inoculated frankfurters was similar ($P > 0{\cdot}95$) with both the surface rinse and stomacher treatment. Because of the ease of sample handling and easier processing of samples by the spiral plater, all further experiments used surface rinse to recover *L. monocytogenes* from the inoculated frankfurters. The acid treatments achieved about a one-half to one log cycle reduction in detectable *L. monocytogenes* initially (zero time) and this pattern remained during storage up to 56 days. The data in Fig. 2 illustrated the effect of acid dips on *L. monocytogenes* on the surface of frankfurters stored at

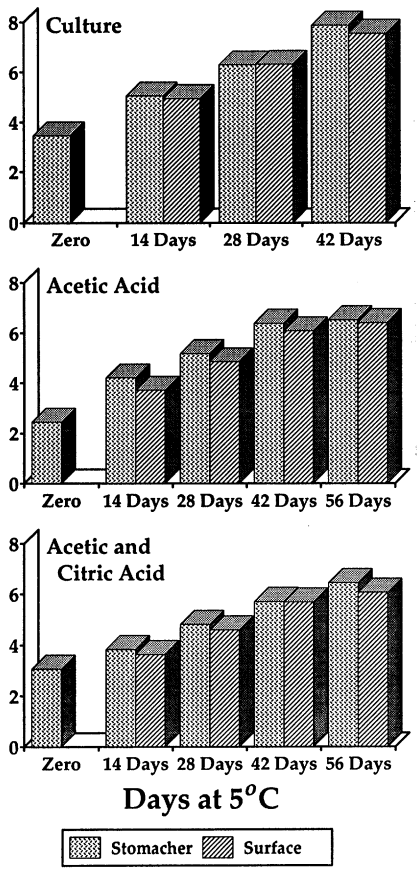


**Fig. 2.** The effect of sample treatment (stomacher vs surface rinse) on the recovery of *L. monocytogenes* from frankfurters after organic acid dips (2% acetic acid and 1% each citric and acetic acids) and storage at 5°C. (Each count represents the average of duplicate plates of duplicate frankfurters; samples plated on MVJ agar.)

5°C. As can be seen, there is a consistent, lower population ($P > 0.95$) of the organism on frankfurters treated with acetic and a mixture of acetic and citric acids compared to untreated frankfurters. When stored at 12°C (mild temperature abuse condition), *L. monocytogenes* inhibition by the acid treatments was lost (data not shown). The *L. monocytogenes* counts on temperature abused, acid dipped frankfurters were not different than those on frankfurters dipped in culture only. Thus, the efficacy of the acid dips cannot counter temperature abuse of a meat product.

*Injury and bacteriostatic vs bacteriocidal action of acid treatment*

The purpose of this portion of the study was to determine if *L. monocytogenes* was injured by the acid treatments and if the order of treatment (acid before or after the *L. monocytogenes* culture) affected the number of *L. monocytogenes* recovered. As defined by Smith and Archer (1988), the amount of injury for *L. monocytogenes* is the difference in count on TA (the permissive medium) vs count on TAS (the restrictive medium). As seen in Table 2, the TA and TAS counts are the same ($P > 0.95$) throughout the course of the storage period including zero time (just after the acid treatment). Also as seen in Table 2, acid dip after addition of the *L. monocytogenes* culture was more effective ($P > 0.95$) than acid treatment before exposure to the bacterium (acid is bacteriocidal), though the data in Table 2 also suggest that an initial acid treatment provided some small residual inhibitory activity (bacteriostatic effect). In this portion of the research, treatment with 2% acetic acid and 1% each acetic and citric acid had similar activity against *L. monocytogenes* on the surface of frankfurters.

Next, both dose and time response studies were performed. In these, frankfurters were surface inoculated as before and then treated for different lengths of time with 2% lactic acid or with different concentrations of acetic or lactic acid for two min. The recovery data are given in Table 1. A general, though not statistically significant, a time response can be seen with the 2% lactic acid treatment. A statistically significant correlation between acid concentration and viable count after treatment was observed for

**Table 2. Effect of plating media (TA vs TAS) and order of treatment on the recovery of *L. monocytogenes* from the surface of frankfurters (recovery by surface rinse technique) during storage at 5°C.**

| DAYS at 5°C | Lm culture only | | Lm[a] culture, then acid | | | | Acid, then Lm culture | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2% acetic acid | | 1% each acetic and citric acids | | 2% acetic acid | | 1% each acetic and citric acids | |
| | TA[b] | TAS[b] | TA | TAS | TA | TAS | TA | TAS | TA | TAS |
| 0 | 4·65[c] | 4·38 | 3·80 | 3·77 | 3·78 | 3·71 | 4·52 | 4·53 | 4·08 | 4·15 |
| 7 | 4·24 | 4·33 | 3·37 | 3·36 | 3·15 | 3·17 | 3·93 | 3·72 | 3·86 | 3·82 |
| 14 | 4·89 | 4·76 | 3·35 | 3·28 | 3·50 | 3·55 | 4·02 | 4·00 | 3·72 | 3·64 |
| 21 | 5·10 | 5·08 | 3·41 | 3·38 | 3·11 | 3·07 | 4·04 | 4·16 | 4·13 | 4·14 |
| 28 | 5·66 | 5·62 | 3·03 | 3·14 | 3·50 | 3·51 | 4·06 | 4·06 | 4·09 | 4·07 |
| 41 | 6·84 | 7·42 | 3·99 | 3·95 | 4·71 | 4·74 | 4·90 | 5·01 | 6·11 | 6·22 |
| 55 | 6·99 | 7·03 | 4·77 | 4·51 | 4·31 | 4·15 | 6·79 | 6·58 | 7·52 | 7·35 |

[a]Lm, *Listeria monocytogenes*.
[b]TA, tryptose agar; TAS, tryptose agar + 5% NaCl; the difference between the two is a measure of the amount of injury.
[c]$\log_{10}$ viable *L. monocytogenes* $g^{-1}$ of frankfurter; average of duplicate plates of duplicate frankfurters.

acetic ($R^2$ = 0·958) and lactic acid ($R^2$ = 0·932). The most destruction occurred with a 2 min dip in 4·5% lactic acid. When the TA and TAS counts for the individual treatments were compared, they were found to be similar ($P > 0·95$), indicating that even with higher concentrations of acid or increased exposure time, *L. monocytogenes* was not injured by the acid treatments.

Based on the destruction of *L. monocytogenes* on the surface of frankfurters with highest concentrations of organic acids, we treated frankfurters with 5% of various acids, vacuum-packaged, and then stored them at 5°C. These data are presented in Fig. 3. In addition to the dramatic decrease brought about by the dip in 5% acid solutions, all the acid treatments restricted the growth of the bacterium during the course of storage. Thus, dips in concentrated (5%) organic acids had similar bacteriocidal and bacteriostatic effects.

In summary, a 2 min acid dip treatment to destroy *L. monocytogenes* on the surface of frankfurters provides one of the first examples of the use of organic acids on a processed meat product. Low levels of acids (1–2%) (Figs 1 and 2) provided a bacteriocidal treatment, with a gradual increase in numbers of the organism recovered from the acid treated frankfurters over increasing storage time. The primary effect of the low level acid dip as bacteriocidal can also be seen in Table 2. For this portion of the study, the order of treatment was varied, acid dip before or after addition of the *L. monocytogenes* culture. As seen in Table 2, the number of organisms recovered from frankfurters acid dipped before inoculation with the culture was higher and their number increased more rapidly during storage. A level of 5% acid (Fig. 3) provided both bacteriocidal and bacteriostatic effects and the number of *L. monocytogenes* recovered from acid treated frankfurters remained low, often at or below the lower limit of detection (21 cfu $g^{-1}$). The greater effectiveness of the 5% acid dip was supported by dose response studies, whether with higher concentrations of

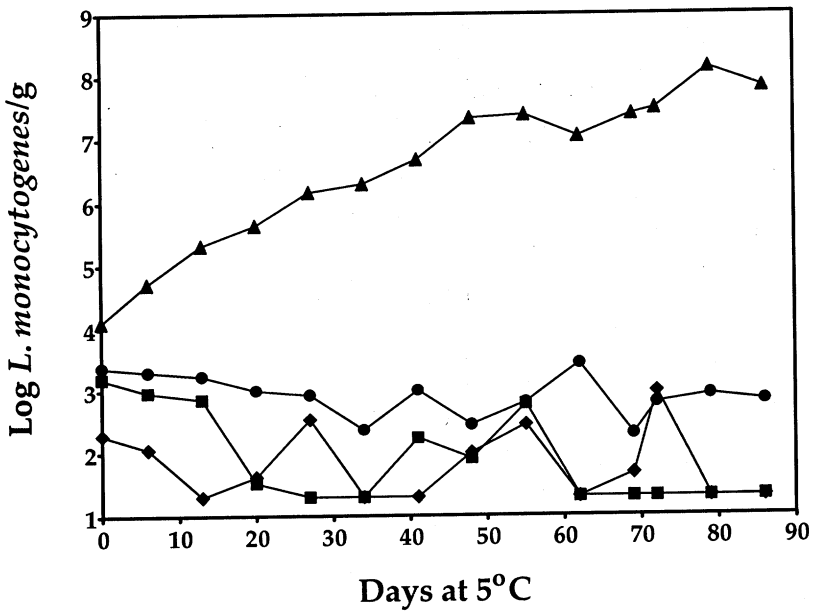


**Fig. 3.** Effect of 5% acid on the recovery of *L. monocytogenes* from the surface of frankfurters: 2 min acid treatment; *L. monocytogenes* recovered by surface rinse. Samples plated on TA; points represent the average of duplicate plates of duplicate frankfurters. A count of 1·3 is at or below the lower limit of detection; ▲, Lm culture; ●, Lm culture and acetic acid; ♦, Lm culture and lactic acid; ■, Lm culture and mixture of 2·5% acetic and 2·5% citric acids.

acid or increased length of dip (Table 1) It is interesting that *L. monocytogenes* did not appear to be injured (using the TA/TAS plating system) by the acid dips (Table 1). The lack of injury was observed immediately post acid dip (zero time of storage) and after extended storage. Overall, incorporation of some type of organic acid treatment, especially with either lactic or acetic acid, could provide additional safety for frankfurters and reduce the risk of lactic or acetic acid, could provide additional safety for frankfurters and reduce the risk of growth of *L. monocytogenes* which might contaminate the frankfurters during the peeling and packaging operations.

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